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# BIOSYNTHESIS OF THE MAJOR CYANOGENIC GLYCOSIDE OF *THALICTRUM AQUILEGIFOLIUM*

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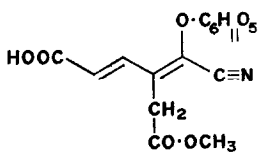
**Key Word Index**—*Thalictrum aquilegifolium*; Ranunculaceae; biosynthesis of cyanogenic glucoside from tyrosine.

**Abstract**—The syntheses and administration of possible precursors of the major cyanogenic glycoside of *Thalictrum aquilegifolium* are described.

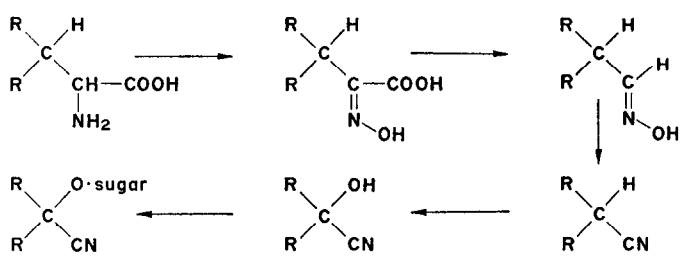
## INTRODUCTION

THE LEAVES of *Thalictrum aquilegifolium* (Ranunculaceae) have been shown to contain three cyanogenic glycosides, one major glycoside accounting for 90% of the cyanogenic content of the plant and two minor glycosides each accounting for 5%.[1] All three glycosides are biosynthetically derived from L-tyrosine.[1] The structures of the two minor glycosides have been shown to be *p*-glucosyloxymandelonitrile and *p*-glucosyloxymandelonitrile-β-D-glucoside respectively. The major glycoside has structure I.[2]




(I)

Tapper *et al.*[3] and Hahlbrock *et al.*[4] have demonstrated that the biosynthetic pathway to cyanogenic glycosides in flax and cherry laurel is via the pathway shown in Scheme 1. We now report feeding experiments designed to demonstrate the operation of this pathway in the L-tyrosine derived major glycoside of *T. aquilegifolium*.




SCHEME 1. PROPOSED BIOSYNTHETIC PATHWAY TO CYANOGENIC GLYCOSIDES FROM THE PARENT AMINO ACID.

[1] D. SHARPLES and J. R. STOKER, *Phytochem.* **8**, 597 (1969).
[2] D. SHARPLES, M. S. SPRING and J. R. STOKER, *Phytochem.* **11**, 3069 (1972).
[3] B. A. TAPPER, E. E. CONN and G. W. BUTLER, *Arch. Biochem. Biophys.* **119**, 593 (1967).
[4] K. HAHLBROCK, B. A. TAPPER, G. W. BUTLER and E. E. CONN, *Arch. Biochem. Biophys.* **125**, 1013 (1968).

## RESULTS

L-Tyrosine-U-$^{14}$C, 4-hydroxyphenylpyruvic acid oxime-2-$^{14}$C, 4-hydroxyphenylacetaldehyde oxime-1-$^{14}$C, 4-hydroxybenzyl cyanide-1-$^{14}$C and 4-hydroxymandelontrile-1-$^{14}$C were administered to cut shoots of *T. aquilegifolium* and the major cyanogenic glycoside isolated and purified by chromatography. The glycoside was assayed by enzymatic hydrolysis and the activity in the hydrogen cyanide liberated measured. The results obtained are shown in Table 1.

TABLE 1. INCORPORATION OF PROPOSED PRECURSORS INTO THE MAJOR CYANOGENIC GLYCOSIDE OF *Thalictrum aquilegifolium*

| Compound administered | Amount (mg) | Specific activity of precursor (dpm/$\mu$M $\times 10^6$) | Glycoside isolated ($\mu$M) | Specific activity of isolated glycoside (dpm/$\mu$M) | Dilution* |
|---|---|---|---|---|---|
| L-Tyrosine-U-$^{14}$C | $4{\cdot}46 \times 10^{-3}$ | 899 | 2·4 | $0{\cdot}39 \times 10^6$ | $2{\cdot}30 \times 10^3$ |
| 4-Hydroxyphenylpyruvic acid oxime-2-$^{14}$C | 19·7 | 97 | 11·2 | $0{\cdot}09 \times 10^3$ | 107 |
| 4-Hydroxyphenylacetaldehyde oxime-1-$^{14}$C | 13·1 | 91 | 11·6 | $0{\cdot}25 \times 10^3$ | 36 |
| 4-Hydroxybenzyl cyanide-1-$^{14}$C | 4·16 | 0·22 | 22·0 | $0{\cdot}12 \times 10^5$ | 18 |
| 4-Hydroxymandelonitrile-1-$^{14}$C | 4·1 | 2·24 | 0·56 | $1{\cdot}98 \times 10^5$ | 11 |

* Dilution $= \dfrac{\text{Specific activity of precursor}}{\text{Specific activity of isolated glycoside}}$.

### *Feeding of Methionine-($^{14}$C)methyl*

The major glycoside of *Thalictrum aquilegifolium* contains a methyl ester grouping. This is probably derived from methionine. To investigate this methionine-($^{14}$C)methyl was fed to cut shoots of *T. aquilegifolium*, the major glycoside isolated and its activity measured. The results obtained are shown in Table 2.

TABLE 2. FEEDING OF METHIONINE-(METHYL)$^{14}$C

| Amount (mg $\times 10^{-2}$) | Activity administered ($\mu$Ci) | Sp. activity of precursor (dpm/$\mu$M $\times 10^7$) | Glycoside isolated ($\mu$M) | Sp. activity (dpm/$\mu$M $\times 10^4$) | Dilution |
|---|---|---|---|---|---|
| 6·0 | 10 | 5·55 | 3 | 1·404 | 3900 |

## DISCUSSION

The results of the feeding experiments indicate that the expected pathway of cyanogenic glycoside biosynthesis is probably operating in the case of the major glycoside of *T. aquilegifolium* (Table 1). This is somewhat surprising in view of the unusual structure of the glycoside. An explanation could be afforded by an *ortho* hydroxylation of the 4-hydroxymandelonitrile, followed by methylation and ring cleavage. Isomerisation would then lead

to the major glycoside. The *ortho* hydroxylation and methylation step is common in higher plants.[5] The incorporation of methionine (Table 2) into the major glycoside appears to support this step. The ring cleavage stage has only been conclusively reported in micro-organisms. Ibrahim *et al.*,[6] however, have suggested that L-tyrosine is metabolized by ring cleavage in higher plants. Recent results by Bough and Gander[7] have shown that dhurrin is not the end point of L-tyrosine metabolism in *Sorghum vulgare* and the evidence suggests that cleavage of the aromatic ring is occurring with partial degradation to $CO_2$.

It seems likely that the cyanogenic glycosides act as a source of HCN which is slowly released and further metabolised to the amino acid asparagine.[8] The HCN could be released either by hydrolysis of the cyanogenic glycoside or by complete breakdown of the cyanogenic glycoside. If the HCN is released by hydrolysis, a build-up of the aldehyde portion of the molecule would be expected. Reay and Conn[9] have shown, however, that *p*-hydroxybenzaldehyde, the aldehyde hydrolysis product of dhurrin, cannot be detected in *S. vulgare* seedlings. Therefore it seems possible that the HCN is released only after cleavage of the aromatic ring and that the major glycoside of *T. aquilegifolium* is a 'trapped' half way stage in the metabolism of 'normal' tyrosine derived glycoside.

## EXPERIMENTAL

*Plant material. Thalictrum aquilegifolium* plants were grown from seed either in the greenhouse or outdoors.

*Radioactive compounds.* L-Tyrosine-U-$^{14}$C, DL-tyrosine-2-$^{14}$C, methionine-($^{14}$C) methyl and potassium cyanide $^{14}$C, were purchased from the Radiochemical Centre, Amersham, Bucks; nitromethane-$^{14}$C from the New England Nuclear Corporation.

*Administration of labelled compounds.* L-Tyrosine-U-$^{14}$C and methionine-$^{14}$C were administered in neutral aqueous solution; the other compounds were dissolved in a drop of MeOH and diluted to *ca.* 10 ml with distilled water before administration. All precursors were administered to cut shoots of the plants, the cut ends being trimmed under $H_2O$ before emersion in the tracer solution. Plants were given 16 hr illumination during the 24 hr metabolic period.

*Analytical methods.* Cyanide was determined by the cyanogen bromide method of Aldridge, after liberation of HCN from the cyanogenic glycoside by either of the two methods described in an earlier paper.[1]

*Isolation of the cyanogenic glycoside.* The major cyanogenic glycoside from *Thalictrum aquilegifolium* was isolated and purified by an analogous method to that described in an earlier paper.[1]

*Determination of radioactivity.* Radioactivity measurements were made in Toluene-Triton X100 solution using an I.D.L. liquid scintillation counter. *n*-Hexadecane-1-$^{14}$C was used as an internal standard.

*Synthesis of labelled precursors.* 4-*Hydroxyphenylpyruvic acid*-2-$^{14}$C. This was synthesized by deamination of DL-tyrosine-2-$^{14}$C using L-amino acid oxidase obtained from *Crotalus adamantus* venom. DL-Tyrosine was shaken for several hr with Sorensen's phosphate buffer pH 7·2. The suspension was filtered, the filtrate assayed for tyrosine by measuring the absorbance of the solution at 275 nm, and comparing with a calibration curve of tyrosine concentration against absorbance at 275 nm. A solution containing 0·36 mg/ml was obtained. To 5 ml of this solution were added DL-tyrosine-2-$^{14}$C (50 $\mu$Ci), *Crotalus adamantus* crude venom (1 mg) and beef liver catalase (5 mg). A few drops of toluene were placed on top of the solution to exclude air and the solution incubated at 30° for 16 hr. After incubation the solution was acidified and extracted with ether. The ethereal extracts were evaporated to dryness *in vacuo* at a temp. not exceeding 30°. The residue of 4-hydroxyphenylpyruvic acid-2-$^{14}$C was diluted with unlabelled 4-hydroxyphenylpyruvic acid (50 mg) and recrystallized from aq. EtOH. 4-*Hydroxyphenylpyruvic acid oxime*-2-$^{14}$C 4-hydroxyphenylpyruvic acid-2-$^{14}$C (13 mg) was dissolved in a small amount of ether and added to a solution of $NH_2OH.HCl$ (100 mg) and $NaHCO_3$ (100 mg) in distilled $H_2O$ (1 ml). The ether was evaporated in a current of air and the solution allowed to stand at room temp. for 18 hr. The solution was acidified, extracted with ether and evaporated to dryness *in vacuo*. The residue of 4-hydroxyphenylpyruvic acid oxime-2-$^{14}$C was recrystallised from ether–*n*-hexane (1:1). 4-*Hydroxybenzyl cyanide*-1-$^{14}$C. 4-Hydroxyphenyl pyruvic acid-2-$^{14}$C (18 mg) and $NH_2OH.HCl$ (10 mg) were suspended in distilled $H_2O$ (2 ml) and refluxed in $N_2$ for 90 min. The solution

[5] J. D. Bu'Lock, in *Biosynthesis of Natural Products*, McGraw-Hill, New York (1965).
[6] R. K. Ibrahim, S. G. Lawson and G. H. N. Towers, *Can. J. Biochem. Physiol.* **39**, 873 (1967).
[7] W. A. Bough and J. E. Gander, *Phytochem.* **10**, 67 (1971).
[8] Y. P. Abrol, E. E. Conn and J. R. Stoker, *Phytochem.* **5**, 1021 (1966).
[9] P. F. Reay and E. E. Conn, *Phytochem.* **9**, 1825 (1970).

was extracted with ether and the residue of 4-hydroxybenzyl cyanide-1-$^{14}$C recrystallised from aq. EtOH. 4-*Hydroxyphenylacetaldehyde oxime*-1-$^{14}$C. This was synthesized from 4-hydroxybenzaldehyde and nitromethane-$^{14}$C according to the method of Kindl and Schiefer.[10] 4-*Hydroxymandelonitrile*-1-$^{14}$C. This was synthesized from 4-hydroxybenzaldehyde and potassium cyanide-$^{14}$C according to the method of Laderberg *et al.*[11]

[10] H. KINDL and S. SCHIEFER, *Montsch. Chem.* **100,** 1773 (1969).
[11] K. LADERBERG, K. FOLKERS and R. T. MAJOR, *J. Am. Chem. Soc.* **58,** 1292 (1936).